

INTRASPECIFIC POLYPLOIDY IN  
*HYPOPTERYGIUM ROTULATUM* (HEDW.) BRID.

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(Plates vi-ix)

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*Synopsis*

Intra specific polyploid races with chromosome numbers of  $n=9$ , 18, ca. 27 and 36 were discovered in plants of *Hypopterygium rotulatum* growing mixed together on the same log at Mt. Wilson, and a population with  $n=18$  was located a mile away.

Cytological behaviour at meiosis was studied, and morphological characteristics of the plants were compared.

Spore mother cells with normal meiosis were found in all chromosome races, but some higher polyploids showed a high proportion of irregularities.

Few morphological differences between the various plant groups were detected. Some increase in the size of spore mother cells and greater variability in size of spores occurred in high polyploids. The leaf cells were more variable and longer in the plants with chromosome numbers of  $n=18$  and 36.

Plants with a chromosome number of  $n=9$  were dioecious while the others were monoecious.

INTRODUCTION

The HYPOPTERYGIACEAE is a well-defined tropical family of mosses, *Hypopterygium* being the largest genus with about 70 species (Brotherus, 1924). *Hypopterygium rotulatum* (Hedw.) Brid. in the sub-genus Eu-hypopterygium, section Pseudo-Tamariscina, is a very variable species (Sainsbury, 1955) evident from the large number of specimens described originally as separate species but now moved into synonymy. The upright almost dendroid gametophytes are produced from a creeping secondary protonema, a number of plants arising along its length. Some of the plants are perennial although new gametophytes arise from the protonema, or new shoots form from the old plants each year. The complanate arrangement of the leaves, and the ventrally placed amphigastria characterize this family. *H. rotulatum* has been recorded in the literature as dioecious (Brotherus, loc. cit.) each female gametophyte bearing several capsules, usually only one from each perichaetium.

Shimotomai and Koyama (1932) obtained a mitotic count of  $n=18$  from gametophyte tissue in the synoecious species *H. japonicum* Mitt. belonging to the sub-genus Eu-Hypopterygium, section Aristifolia. No other chromosome numbers have been recorded for this family.

MATERIALS AND METHODS

The specimens of *Hypopterygium rotulatum* (Hedw.) Brid. were collected from two areas about one mile apart at Mt. Wilson, N.S.W. at an altitude of about 3,200 ft. in shaded rainforest. The majority were growing on a log which was covered for more than six feet on its sides and underneath by *H. rotulatum* and *Pterygophyllum dentatum*. Plants of *Hypopterygium* were growing on rotting logs, earth and rocks on the banks of a stream. As some variation in the sizes of plants was noticed, groups of plants were kept separate. Specimens were

kept in glass dishes for two to three weeks in the laboratory until meiosis was completed, then plants were pressed. These voucher specimens have been retained in the Ray Herbarium, Botany Department, University of Sydney (for numbers see Table 1).

Smear preparations of meiotic stages in spore mother cells were obtained using the method outlined in Ramsay (1964, 1966). Drawings and photomicrographs were made using Zeiss microscope equipment and phase contrast microscopy as in Ramsay (1966) and are reproduced here at a magnification of  $\times 2,700$  for drawings and at quoted magnifications for the photomicrographs.

### OBSERVATIONS

In Table 1 it can be seen that a series of chromosome numbers was obtained for the 12 different groups of plants of *Hypopterygium rotulatum* examined. The chromosome numbers of  $n=9$ , 18 ca. 27 and 36 represent a polyploid series in plants growing in the same locality and in close proximity.

The results deal separately with the chromosome numbers and behaviour at meiosis, and with the comparison of taxonomic features of plants from each of the chromosome groups.

TABLE 1  
*Chromosome numbers in Hypopterygium rotulatum*

Date Collected	Voucher* Number	Sex	Chrom. Number	Locality	Figures	Plates
16-4-64	8/64	D	9	Log, waterfall	1	VI.1
6-9-65	36/65	D	9	Log, waterfall	2-3	VI.2
16-4-64	8a/64	M	18	Log, waterfall	4, 6	VI.3
1-5-64	33/64	M	18	Happy Valley	5	VI.4
	33a/64					
	33b/64					
1-5-64	22/64	M	ca. 27	Rocks, waterfall	7-9	—
16-4-64	8d/64	M	ca. 27	Log, waterfall		VI.5-11
16-4-64	8e/64	M	36	Log, waterfall	10-11	VII.1-3
16-4-64	8b/64	M	36	Log, waterfall	—	—
16-4-64	8c/64	M	36	Log, waterfall	—	VII.4
16-4-64	8f/64	M	36	Log, waterfall	—	VII.5-8
16-4-64	16/64	M	36	Rocks, waterfall	—	—

M=monoecious. D=dioecious. All specimens were collected from Mt. Wilson (3,200 ft.) in New South Wales.

\* Specimens deposited in the Ray Herbarium, Botany Department, University of Sydney.

### CYTOLOGICAL BEHAVIOUR

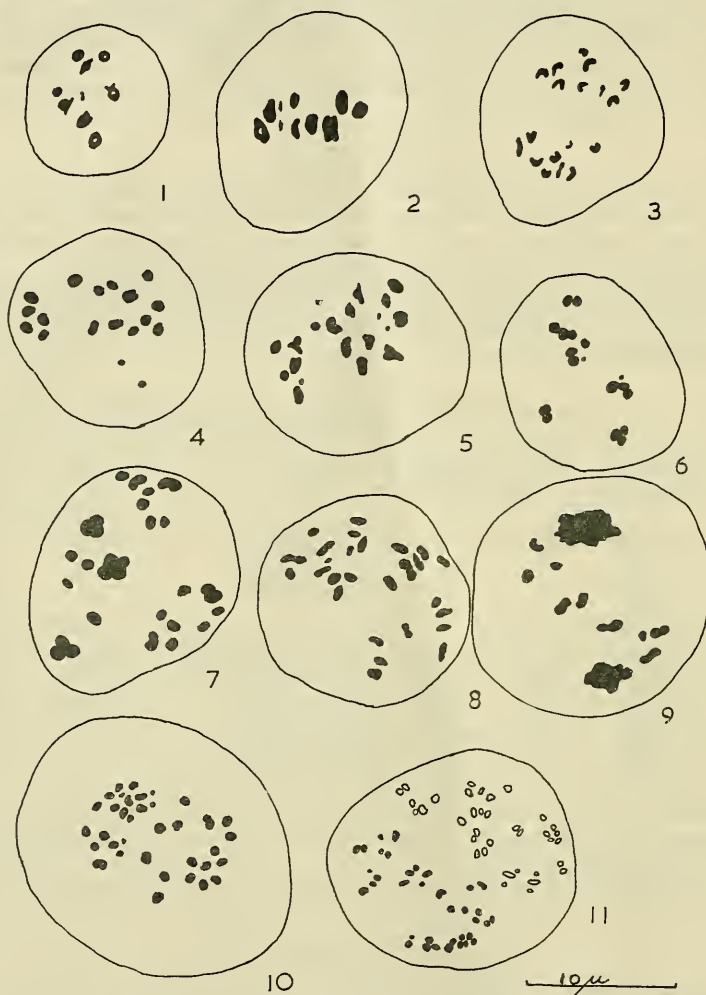
*H. rotulatum* (8/64, 36/65)  $n=9$  (Text-figs 1-3. Plate VI, 1-2).

Both these dioecious populations were collected from the same log, the former in April, 1964, the latter in September, 1965 (Table 1). A chromosome number of  $n=9$  was observed at Metaphase I of meiosis (Text-figs 1-2. Plate VI, 1-2). Eight of the bivalents were of almost uniform size, but one was smaller and disjoined precociously in the majority of cells (Text-fig. 2). Its size and precocious behaviour are similar to that found in a number of other mosses (Vaarama, 1953, 1956, Steere, 1954, *etc.*, Yano, 1957, Khanna, 1960, *etc.*, and Ramsay, in a number of Australian mosses unpubl.).

At Anaphase I, nine chromosomes were observed to pass regularly to each pole (Text-fig. 3). Tetrad formation was normal and no lag of chromosomes was seen.

Heteropycnosis or precocious staining of part of the nucleus, usually in the form of an open ring, was visible at early prophase of meiosis. However, it was much less pronounced than in many other mosses *e.g.*, *Bryum*, *Rhizogonium*,

*etc.* (Ramsay, unpubl.). Even in dioecious species no evidence was found of a large bivalent which separated precociously and could be related to the possible sex chromosomes described in some other mosses *e.g.*, *Macromitrium* (Ramsay 1966), *Pogonatum* (Yano, 1955, 1957), *etc.*



Meiosis in spore mother cells of *Hypopterygium rotulatum*  $\times 2,700$ . 1, 8/64 M-I,  $n=9$ , note small bivalent; 2-3, 36/65. 2, M-I,  $n=9$ , note small bivalent separating precociously; 3, A-I small chromosome distinct at each end; 4, 8a/64 M-I,  $n=18$ , note two small bivalents; 5, 33a/64 M-I,  $n=18$ , note similarity to fig. 1 but twice the number; 6, 8a/64 M-I, secondary association of bivalents; 7-9, 22/64.  $n=27$ ; 7, multivalents or secondary association of some bivalents; 8, 27 bivalents at M-I; 9, T-I, note 9 lagging univalents or bivalents; 10-11, 8e/64,  $n=36$ ; 10, M-I, 36 bivalents visible including four small ones; 11, A-I.

*H. rotulatum* (8a/64, 33/64, 33a/64, 33b/64)  $n=18$  (Text-figs 4-6. Plate VI, 3-4).

The first of these populations was collected on the same log with the previous ones, but the other was found about one mile downstream in Happy Valley (Table 1). Both had 18 bivalents at metaphase of meiosis, consisting of 16 similar in size, and two small precociously disjoining ones. Comparison of

bivalents in Plate VI, 1-2 ( $n=9$ ) and Plate VI, 3 ( $n=18$ ) suggests that the latter is made up of  $2 \times 9$  chromosomes. The gametophytes of these must therefore be diploid.

Although the populations had the same chromosome number, it was noticed that in 8a/64 secondary association with two or more bivalents associated together at metaphase I was common in dividing cells (Plate VI, 3). During meiosis irregularities observed included univalents left out at telophase I and clumping of groups of chromosomes off the metaphase I plate. Chromosome balance was obviously unstable in this group of plants.

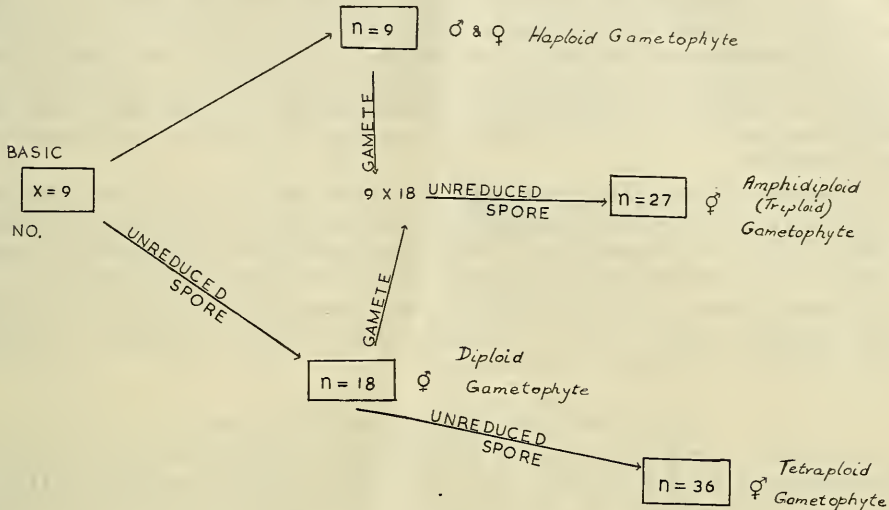


FIG 12

Possible origin of chromosome races in *Hypopterygium rotulatum*.

However, meiosis in 33/64, 33a/64 and 33b/64 was normal and a very small percentage of cells behaved irregularly. All the plants in this chromosome group on further examination were found to be monoecious, the antheridia being borne in separate perichaetia in the axils of leaves just below the archeogonial 'inflorescences'.

The mitotic chromosomes examined by Shimotomai and Koyama (1932) in the synoecious species *H. japonicum* consisted of 16 large and two small chromosomes, and compare in relative sizes to those in *H. rotulatum* with  $n=18$ .

*H. rotulatum* (22/64, 8d/64)  $n \approx 27$  (Text-figs 8-9. Plate VI, 5-11).

Meiosis in the spore mother cells of the above groups of plants revealed approximately 27 bivalents at metaphase I. Some had been collected from the same log at Mt. Wilson (Table 1). Only an approximate chromosome count could be obtained, for although some cells had  $n=27$  (Text-fig. 8. Plate VI, 6) clumping, irregularities and multivalent formation or secondary association made it difficult to interpret divisions (Plate VI, 7-10). A maximum of 32 including possible univalents or dissociated bivalents was found and none gave a number as high as 36. Some side views of metaphase I showed a regular arrangement of bivalents on the equator, and anaphase I revealed no lagging chromosomes in a number of the spore mother cells, but others (Text-fig. 9. Plate VI, 11) had as many as nine univalents or bivalents left out at end of anaphase I. Normal tetrads with four equal nuclei were produced in some

spore mother cells, although many contained large and small nuclei, or chromosomes lying loosely in the cytoplasm even after the spores had been differentiated.

*H. rotulatum* (8e/64, 8b/64, 8c/64, 8f/64, 16/64)  $n=36$  (Text-figs 10-11. Plate VII, 1-8).

Table 1 shows that some of these plants also came from the same location at Mt. Wilson. Their chromosome number was found to be  $n=36$ .

Meiosis was almost regular in 8e/64 although some secondary association was noticed at metaphase I. Separation of chromosomes at anaphase I proceeded normally and lag was infrequent. Tetrad formation appeared normal.

In other populations behaviour at meiosis was very irregular. Secondary association of bivalents was common (Plate VII, 4-6), bivalents and univalents lay off the metaphase I plate, lag at anaphase I (Plate VII, 7) was frequent and unequal distribution of chromosomes to nuclei at telophase I and telophase II resulted in irregular tetrads with only three nuclei, or with more than four usually unequal in size. It is unlikely that any viable spores could result from such behaviour.

Some breakdown at meiosis might have been due to the excessive numbers of chromosomes or bivalents struggling for position on the equator at metaphase, so that groups of chromosomes, unable to manage this successfully, formed themselves into nuclei containing univalents as well as whole bivalents.

Heteropycnosis at early prophase in the polyploids was identical with that in the haploids (Plate VII, 1), no comparable increase in amount or number of heteropycnotic regions being observed.

#### COMPARISON OF TAXONOMIC CHARACTERS

A summary of the features of the plants examined is set out in Table 2. Photographs of the leaves and cells of the leaves are found in Plates VIII and IX. The leaves illustrated were taken from side shoots between the middle and the base of the shoot. The gametophyte plants used for these measurements bore several sporophytes, at least two having been examined cytologically. Photomicrographs of cells were taken towards the mid-leaf near the end of the nerve.

So that comparison of gametophyte characters could be made in plants with different chromosome numbers, it was assumed that the chromosome number of the gametophyte corresponded to the haploid chromosome number (i.e.  $n=9$  or 18, 27, 36, *etc.*) obtained at meiosis in the attached capsules. Since all capsules on each plant used had the same chromosome number it seemed reasonable to suppose this to be true, in the absence of mitotic counts. Some of the possible variations in the chromosome numbers of gametophytes giving rise to capsules containing the various chromosome numbers are suggested in the discussion, and the need for actual mitotic counts of gametophyte cells has already been emphasized. Some comments on the methods used for obtaining the measurements are described below.

*Height of Gametophyte plants.* The total height included distance from ground level to the apex of the leafy branches but did not include the sporophyte

*Leaf size.* The average lengths (not including the point) and breadths (in the mid-leaf) of ten leaves and amphigastria were obtained.

*Cell size.* Twenty cells were examined from areas corresponding to the photographs in Plate IX.

*Spore mother cells and spore size.* Diameters of 20 spores and 20 spore mother cells were measured. Since the spore mother cells were squashed, an area in which the cells were evenly spread was chosen for measurements to give more real comparisons. It was not possible to calculate volumes since the diameter of the squashed cells did not correspond to the original diameter.

TABLE 2  
*Comparison of morphological characteristics of Hypopterygium rotulatum*

Voucher Number	Chrom. Number n=	Height of gametophyte cm.	Sex	Leaf Size mm. l × b	Amph. Size mm. l × b	Cell Size		Diam. S.M.C. μ	Spore Size μ
						Leaf μ l × b	Amph. μ l × b		
8/64	9	2-2.5	♂ and ♀	1.0 × 0.7	0.5 × 0.5	3.8 × 2.4 ± 0.05	3.7 × 2.3	0.99	12-14
33/64	18	2-2.8	♂ +	1.1 × 0.7	0.6 × 0.6	4.9 × 2.6 ± 0.4	3.7 × 2.4	1.61	11-12
22/64	27	1.5-2.0	♂ +	0.93 × 0.7	0.5 × 0.5	3.7 × 2.4 ± 0.2	3.8 × 2.5	1.44	10-13
8f/64	36	1.8-2.0	♂ +	1.05 × 0.8	0.6 × 0.6	4.4 × 2.6 ± 0.5	4.25 × 2.0	1.65	10-16

Amph. = amphigastria. S.M.C. = spore mother cells. l = length. b = breadth.

The results set out in Table 2 give some indication of the features most likely to be of use in further investigations, particularly of gametophytes from which mitotic counts could be obtained. Although the leaves and amphigastria showed little difference in gross size in the various groups of plants studied, the leaf cells in particular showed increase in length and greater variation in plants with chromosome numbers of  $n=18$  and  $36$ . In Plate IX the numbers of cells in a given area appear to be greater in the haploid and triploid and fewer but larger in the diploid and tetraploid. It has been pointed out by Lewis (1961) that decrease in numbers of cells may account for apparently little change in overall size of some polyploids.

The length of setae and size of capsules was similar in all chromosome races, and measurements were not therefore included in Table 2. However, a clear difference in the sizes of spore mother cells can be seen in Table 2, and by comparison of the Text-figures and Plates of the various chromosome forms. Spore size data shows a greater variation in the sizes of spores in polyploids. It is interesting to note that the characters examined in plants with a chromosome number of  $n=\text{ca. } 27$  (22/64) were much closer to the 'original haploid' (i.e. plants with  $n=9$ ) than to the others.

It appears, therefore, that increase in the length of the cells of the leaf, an increase in the size of spore mother cells and a greater range of variation in the size of spores are features which reflect the increase in chromosome numbers in the various plants. Other characters remained much more stable throughout the groups.

#### DISCUSSION

*Polyploidy in Mosses.* Before chromosome numbers in mosses had generally been investigated polyploid gametophytes were produced experimentally by apospory (É. and Ém. Marchal, 1911) in *Bryum caespitium* and *B. argenteum*. Later Wettstein (1923-1924, 1928) produced a series of polyploid gametophores in *Bryum* by treatment of the protonema, or of the spore mother cells at meiosis in *Funaria*. Auto- and amphidiploid races were produced in several mosses e.g. *Funaria hygrometrica*  $n=14$  to  $28$  and  $56$ ; the polyploid population of *Bryum caespitium*  $n=10$  was called *B. corrensii* with  $n=20$ ; *Amblystegium serpens* from  $n=12$  to  $24$ ; and *Physcomitrium pyriforme* from  $n=18$  to  $36$  and  $72$ .

Increasing numbers of naturally occurring inter- and intra specific polyploids are being reported as the chromosome numbers of mosses from many parts of the world become known (Wylie, 1957; Steere and Anderson, 1954; Khanna, 1960; Yano, 1957 *a, b, c, etc.*). Examples include *Atrichum undulatum* (Hedw.) P. Beauv. (Lowry, 1954) with  $n=7, 14$  and  $21$ , *Mnium* spp. (Lowry 1948)  $n=6, 12$  *Tortula princeps* De Not.  $n=12, 24+1$  and  $36+2$  (Steere, 1954; Steere *et al.*, 1954) *Philonotis socia* Mitt. (Yano, 1957*a*) with  $n=6$  and  $12$  and *Octoblepharum albidum* Hedw. with  $n=13$  and  $26$  (Khanna, 1960). They represent species from many families. *Octoblepharum albidum* was analyzed by Khanna (1960) who found no qualitative differences between the plants in external characteristics such as leaf size, size of leaf cells, *etc.* Some differences in spore size were noted, although overlapping occurred, and from his drawings it appears that the spore mother cells were larger in the polyploid.

The majority of the examples of intraspecific polyploidy represent different populations in different localities e.g. *Tortula mucronifolia*  $n=12$  and  $24$ , *Drepanocladus uncinatus*  $n=12$  and  $24$ , *Distichium capillaceum*  $n=14$  and  $28$  (Anderson and Crum, 1958). The spore mother cells of the latter were found to be larger in the diploid, although the spores were similar in size.

One particularly interesting series of interspecific polyploids exists in the genus *Bryum*, where the chromosome numbers of  $n=10, 20, 30, 40$  and  $50$  were discovered in Alaskan species by Steere (1954).

Lewis (1961) has summarized the work of Wettstein (1940) on the relationship of cell size and volume in artificially induced auto- and allo-polyploids. "Generally an increase in size of cells, organs and individuals follows recently induced polyploidy but the extent of such increases varies. The effect is not uniform over the entire polyploid range, cell size usually decreasing, or ceasing to increase especially in the more hybrid polyploids" (Lewis, loc. cit.). In the auto-polyploids of *Physcomitrium pyriforme* cell size was shown to increase with increase in chromosome number, particularly in the leaf. A negative correlation was found between the initial number of chloroplasts and their number, following doubling of the chromosome numbers (Lewis, 1961). The number of cells in the polyploid organism may be less, so that increase in cell size may not lead to an increase in the size of the organ (Darlington from Lewis, 1961).

A significant feature of size relations of polyploids to normal plants is that recently produced polyploids may show distinct variations but natural polyploids do not. Wettstein (1937) observed that in an artificial diploid strain of *Bryum caespitium* which he called *B. corrensii*, the size of cells and other morphological characters were much larger in the newly produced polyploid, but gradually decreased over a period of 11 years until they had reverted to the size of the original haploid cells etc. During the same period meiosis and spore production also became normal, although very irregular in the newly produced diploid. The stabilization of cell size and other characters and the establishment of normal spore production were found in the original plants after 11 years, in clones produced vegetatively during this time, and in successive generations produced from the first fertile spores.

Khanna (1960) suggested that polyploid populations of *Octoblepharum albidum* were in a state of reversion to haploid size since variations in the cell size, spore size etc. between the diploid and haploid plants were not constant. Meiotic behaviour in auto-polyploids of maize, studied in detail by Giles and Randolph (1951), showed a reduction in multivalent frequency at meiosis in successive generations, until within 10 years meiotic behaviour had reverted to normal.

Apart from influencing the autosome number, polyploidy must also affect sex balance in dioecious species, whether sex is determined by a sex chromosome or a gene complex on autosomes. Doubling of the chromosome number can occur in several ways in mosses such as regeneration from gametophyte cells in which breakdown of mitosis doubles the chromosome number, by apospory from cells of the seta or capsule, or by reconstituted nuclei in spore production at meiosis. The latter would result in the production of monoecious plants.

It has been found generally that polyploids are monoecious (Mehra and Khanna, 1961; Anderson, 1964, for summaries) and apospory is probably the most important factor in the evolution of polyploidy in mosses. The chromosomes of such polyploids would have more affinity for their exact replicas than related chromosomes which may bear other alleles, and secondary association of bivalents might occur more frequently than multivalent formation.

#### *Polyploidy in Hypopterygium rotulatum.*

The area from which the plants of this species was collected has been re-visited at frequent intervals in an attempt to repeat the results, and to study the whole population in greater detail. Unfortunately, 1965 and 1966 have been drought years with unusually low rainfalls, particularly in the summer months. In 1965 plants did not regenerate in large numbers, although protonema and old and immature plants were present in the winter months. Very few capsules were produced, and the only chromosome number obtained was  $n=9$  in a few capsules in September (earlier investigations had been carried out in April, 1964). The population is again showing considerable growth and archegonia and antheridia are being produced. Dioecious and monoecious forms are

present, although only a few capsules have been found in 1966, but meiosis has not been detected. Drought could again influence fertilization in these plants. The population is obviously maintained by vegetative means.

The necessity for water to carry the sperm to the ovum, especially in dioecious species, is a controlling factor for fertilization. Monoecious forms which allow self fertilization would be able to overcome this if a thin film of water were present, and the antheridia and archegonia were placed close together and ripened simultaneously. The genetic disadvantage of selfing is the establishment of strict homozygosity. Isolating mechanisms such as protandry, or self incompatibility with their genetic advantages of outbreeding, are disadvantages if fertilization depends rigidly on sufficient water at the correct time.

Taxonomic descriptions of *H. rotulatum* state that it is dioecious, whereas the majority of plants examined in this study were found to be monoecious.

The populations which showed almost normal meiosis such as 33/64, 8e/64 and also produced normal looking spores may have resulted from self fertilizations. Cross fertilization in an area where such a variety of chromosome numbers were present would be particularly hazardous. Some of the possible crosses within such a population are set out in Table 3.

TABLE 3

	Egg	Sperm	Expected meiotic behaviour
n=9	9	×	9 normal pairing
	9	×	18 9 pairs, 9 univalents, or trivalents
	9	×	27 9 pairs, 18 univalents or multivalents according to affinities
	9	×	36 9 pairs, 27 multivalents or univalents
n=18	18	×	18 pairs, normal behaviour
	18	×	9 9 pairs, 9 univalents or multivalents

The ovum may have some control over which sperm are acceptable. Other controls may be affected by the genetic affinities of the two nuclei. In some plants sporophytes reached the stage of differentiation into capsules, or almost to formation of the spore mother cells, then shrivelled up. No environmental factor causing this could be found, as such plants were growing among those with well-developed capsules. They may have been the result of irregular fertilizations.

Wettstein (1940) mentions that *Bryum corrensii* did not produce sporophytes, which matured to form capsules and spores, for several years. During the first few years after the development of this experimental polyploid, embryo sporophytes developed only for a short time and capsules were not formed. Within 11 years normal sporophytes were regularly produced.

From these studies it is clear that polyploid gametophytes must exist among the haploid plants of *Hypopterygium rotulatum*, although mitotic counts are needed for confirmation. A scheme setting out the possible origins of the various chromosome numbers is set out in Text-figure 12.

Evidence for such a scheme comes from the apparent duplication of complements in plants with n=9 and those with n=18 most obvious in the doubling of the number of minute bivalents. The four small bivalents in plants with n=36 (8e/64) points to a further doubling from n=18. All the polyploids are monoecious, while haploids are dioecious, as would be expected if the new numbers had resulted from failure of reduction in spores at meiosis. Plants with a chromosome number of n=27 could result from fusion of gametes with chromosome numbers of n=9 and 18, followed by reconstitution at meiosis, giving rise to unreduced spores

The results of this investigation appear to be the first in which a population of mosses growing in close proximity show such high grades of intra specific polyploidy. The levels of polyploidy correspond to tetraploids, hexaploids, and octoploids in Angiosperms.

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## EXPLANATION OF PLATES VI-IX.

## Plate vi

*Hypopterygium rotulatum*, meiosis in spore mother cells,  $\times 2,200$ . 1-2, M-I,  $n=9$ , note small bivalent disjoined in 2. 3-4, M-I,  $n=18$ . 3, secondary association in  $8a/64$ . 4, 18 bivalents in  $33/64$  includes two small ones (tiny spots are excess stain). 5-10,  $n=ca. 27$  various stages of meiosis. 5, premetaphase stage before chromosomes are fully contracted, multivalents indicated by arrows. 6, M-I,  $ca. 27$  bivalents showing. 7-8, side view M-I, showing bivalents left off the plate. 9, clumping of chromosomes at M-I. 10, secondary association or multivalents at M-I. 11, late A-I, showing lagging chromosomes, one bivalent in centre not disjoined.

## Plate vii.

*Hypopterygium rotulatum*, meiosis in spore mother cells of populations with  $n=36$  chromosomes,  $\times 2,200$ . 1, heteropycnosis in polyploid. 2-3, M-I,  $n=36$ , fairly regular arrangement on the metaphase plate. 4-6, M-I, secondary association, clumping in five and six, while a ring is clear in four. 7, T-I, lag of univalents. 8, tetrad of spores, note chromosomes left in cytoplasm.

## Plate viii.

*Hypopterygium rotulatum*, leaves from plants with different chromosome numbers,  $\times 30$ . 1' plants with  $n=9$  ( $8/64$ ). 2, plants with  $n=18$  ( $33/64$ ). 3, plants with  $n=27$  ( $22/64$ ). 4' plants with  $n=36$  ( $8f/64$ ).

## Plate ix.

*Hypopterygium rotulatum*, cells of leaves in Plate VIII,  $\times 350$ . 1, cells from plants with  $n=9$  ( $8/64$ ). 2, cells from plants with  $n=18$  ( $33/64$ ). 3, cells from plants with  $n=27$  ( $22/64$ ). 4, cells from plants with  $n=36$  ( $8f/64$ ).